BARD Final Scientific Report
Cover Page

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Project Title: Thermotolerance acquisition in broiler chickens by temperature conditioning early in life.

Investigators	**Institutions**
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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Thermal conditioning, thyroid hormones, growth hormone, stress hormones, sensible heat loss, heat production, metabolic fuel, hypothalamus gene expression, liver gene expression.

Abbreviations commonly used in the report, in alphabetical order**:**
PO/AH – preoptic anterior hypothalamus; TC – thermal conditioned

Budget: IS: $ 183,660 US: $ 101,310 Total: $ 284,970

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	2		1	3
Submitted, in review, in preparation		1	1	2
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts	2		2	4
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

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Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		1	1	2
Longer Visits (Sabbaticals)				

Description of Cooperation:

The optimal temperature, age and duration of thermal conditioning was done in Israel during the 1st year, and was used for further experiments in both countries. Hormones and metabolic fuel analysis were done mainly in the U.S.A. while heat production, heat loss and water balance were done mainly in Israel. The experiments were coordinated at the levels of planning, evaluation, reporting and manuscripts writing. The I.P. partially participated in one of the 2nd year experiments in the state.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

The research on thermotolerance acquisition in broiler chickens by temperature conditioning early in life was focused on the following objectives: a. To determine the optimal timing and temperature for inducing the thermotolerance conditioning processes and to define its duration during the first week of life in the broiler chick. b. To investigate the response of skeletal muscle tissue and the gastrointestinal tract to thermal conditioning. This objective was added during the research, to understand the mechanisms related to compensatory growth. c. To evaluate the effect of early thermoconditioning on thermoregulation (heat production and heat dissipation) during 3 phases: (1) conditioning, (2) compensatory growth, (3) heat challenge. d. To investigate how induction of improved thermotolerance impacts on metabolic fuel and the hormones regulating growth and metabolism.

Recent decades have seen significant development in the genetic selection of the meat-type fowl (i.e., broiler chickens); leading to rapid growth and increased feed efficiency, providing the poultry industry with heavy chickens in relatively short growth periods. Such development necessitates parallel increases in the size of visceral systems such as the cardiovascular and the respiratory ones. However, inferior development of such major systems has led to a relatively low capability to balance energy expenditure under extreme conditions. Thus, acute exposure of chickens to extreme conditions (i.e., heat spells) has resulted in major economic losses. Birds are homeotherms, and as such, they are able to maintain their body temperature within a narrow range. To sustain thermal tolerance and avoid the deleterious consequences of thermal stresses, a direct response is elicited: the rapid thermal shock response – thermal conditioning. This technique of temperature conditioning takes advantage of the immaturity of the temperature regulation mechanism in young chicks during their first week of life. Development of this mechanism involves sympathetic neural activity, integration of thermal information in the hypothalamus, and buildup of the body-to-brain temperature difference, so that the potential for thermotolerance can be incorporated into the developing thermoregulation mechanisms.

Thermal conditioning is a unique management tool, which most likely involves hypothalamic thermoregulatory threshold changes that enable chickens, within certain limits, to cope with acute exposure to unexpected hot spells. Short-term exposure to heat stress during the first week of life (37.5±1ºC; 70-80% rh; for 24 h at 3 days of age) resulted in growth retardation followed immediately by compensatory growth, which resulted in complete compensation for the loss of weight gain, so that the conditioned chickens achieved higher body weight than that of the controls at 42 days of age. The compensatory growth was partially explained by its dramatic positive effect on the proliferation of muscle satellite cells which are necessary for further muscle hypertrophy. By its significant effect of the morphology and functioning of the gastrointestinal tract during and after using thermal conditioning. The significant effect of thermal conditioning on the chicken thermoregulation was found to be associated with a reduction in heat production and evaporative heat loss, and with an increase in sensible heat loss. It was further accompanied by changes in hormones regulating growth and metabolism. These physiological responses may result from possible alterations in PO/AH gene expression patterns (14-3-3ε), suggesting a more efficient mechanism to cope with heat stress.

Understanding the physiological mechanisms behind thermal conditioning step us forward to elucidate the molecular mechanism behind the PO/AH response, and response of other major organs.

The thermal conditioning technique is used now in many countries including Israel, South Korea, Australia, France, Ecuador, China and some places in the USA. The improvement in growth performance (50-190 g/chicken) and thermotolerance as a result of postnatal thermal conditioning, may initiate a dramatic improvement in the economy of broiler's production.

Achievements

Significance of main scientific achievements and innovations

Thermal conditioning was found to improve both performance and thermotolerance. According to our knowledge, it is the first time that these two contradictive processes (Emmans and Kyriazakis, 2000) can be improved.

Thermal conditioning is a unique management tool**,** which most likely involves hypothalamic thermoregulatory threshold changes that enable chickens, within certain limits, to cope with acute exposure to unexpected hot spells. Short-term exposure to heat stress during the first week of life (37.5±1^{o}C; 70-80% rh; for 24 h at 3 days of age) resulted in growth retardation followed immediately by compensatory growth, which resulted in complete compensation for the loss of weight gain, so that the conditioned chickens achieved higher body weight than that of the controls at 42 days of age (Yahav and McMurtry, 2001). The higher body weight coincided with higher feed intake**,** but with no significant effect on feed efficiency.

A possible mechanism behind the growth inhibition and acceleration has been suggested by the recent results of this research (Halevy et al., 2001). Thermal conditioning in young chicks elicits an increase in the proliferation of satellite cells. These cells are necessary for further muscle hypertrophy. In thermally conditioned 3-day-old chicks, an immediate increase in the number of satellite cells was observed, to levels that were significantly higher than that in the control chicks. This was accompanied by a marked induction of insulin-like growth factor-I (IGF-I). This is the first time that response of satellite to thermal treatment is demonstrated.

The effect of thermal conditioning on gastro-intestinal tract development (Uni et al. 2001) was also elucidated. Several immediate effects were observed including lowered T_3 levels, reduced feed intake, and depressed enterocyte proliferation and brush-border membrane (BBM) enzyme activity. A second series of effects, which were observed 48 h post-treatment, included**,** elevated T_3, increased feed intake, increased enterocyte proliferation, and higher expression and activity of BBM enzymes. The association between, ambient temperature, feed intake, growth rate, and plasma T_3 levels was reflected in the structure and function of the intestinal tract. These results suggest that thermal conditioning at an early age influences T_3 concentrations, which in turn alter the intestinal capacity to proliferate, grow and digest. These changes modulate the intestinal tract for compensatory growth commencing 48 h following thermal treatment.

The concentration of circulating T_3, which is an important growth promoter (McNabb and King, 1993) and is positively correlated with feed intake in chickens and turkeys (May,

1978; Klandorf and Harvey, 1985; Yahav, 2000; Yahav et al., 1995, 1996, 1998) also plays a major role in controlling the growth pattern of thermally conditioned chickens. The accelerated compensatory growth, occurring immediately after thermal conditioning**,** was accompanied by the relatively high concentrations of plasma T_3**.** These elevated levels were detected only during the compensatory period, after which they declined to relatively low levels compared with those of the control chickens. This strongly suggests that the depressed T_3 levels facilitates relatively low heat production during exposure to heat stress. It seems, therefore, that plasma T_3 plays an important role in both growth inhibition and accelerated growth.

The results in these studies demonstrated a significantly higher stress status in the control chickens during the heat challenge phase compared to thermal conditioned birds. This is based on the corticosterone (Puvadolpirod and Thaxton, 2000), glucagon (Freeman, 1976, 1980, 1982) and IGF II (McMurtry et al., 1998) response to stress.

It can be concluded therefore, that the control chickens were under a higher stress condition than the treated chickens, as was also demonstrated by their rate of mortality that amounted during the challenge to 50% compared with 24% of the TC chickens. The significant decline in insulin concentration in both treatments during thermal challenge can be related to its thermogenic effect (Piolino et al., 1990). The ability of the TC chickens to maintain lower body temperature (Yahav and Hurwitz, 1996), to reduce evaporative heat loss, to increase sensible heat loss, suggested a more efficient ability to cope with heat stress as a result of the early in life thermal conditioning.

The increase in the expression of the gene14-3-3ε, which is strongly implicated with neuronal growth and development, may shed light on the influence of thermal conditioning on the PO/AH - the center of body temperature control.

The significant effect of thermal conditioning chicks on their ability to improve thermotolerance was found to be associated with a reduction in heat production and evaporative heat loss, with an increase in sensible heat loss. These physiological responses may be possible as a result of alterations in PO/AH gene expression patterns, suggesting a more efficient mechanism to cope with heat stress.

It can be concluded that the thermoregulatory and growth mechanisms of thermal conditioned chickens was elucidated, leaving the gene expression involvement to be studied to complete the knowledge involved in this unique processes.

Agriculture or economic impacts

The morbidity and mortality of the genetic selected broilers as a result of elevated environmental temperature, causes large economy losses. This research provides a management tool that reduces the mortality from heat stress by up to 50% (Yahav and McMurtry, 2001). It has further significant economy aspect because most of the mortality is during the aged phase, i.e. above 4 weeks of age, when the main investment in the flock was already done.

The other main aspect is related to the elevated body weight at marketing. In all thermal conditioning experiments, body weight of thermal conditioned chicken was higher by 50 to 190 g in comparison to control chickens at marketing age. The price of 1 kg broiler ranged between 3.2 to 4.5 NIS, depends on the market response. Increase of 50 g body weight per chicken can significantly affect the farmer income.

Details of cooperation

The optimal temperature, age and duration of thermal conditioning was done in Israel during the 1st year, and was used for further experiments in both countries

Hormones and metabolic fuel analysis were done mainly in the U.S.A. while heat production, heat loss and water balance were done mainly in Israel.

The experiments were coordinated at the levels of planning, evaluation, reporting and manuscripts writing.

The I.P. partially participated in one of the 2nd year experiments in the state.

List of publications

Halevy O., Krispin A., Leshem Y., McMurtry J.F. and Yahav S. (2001). Early age heat stress accelerates skeletal muscle satellite cell proliferation and differentiation in chicks. Am. J. Physiol. 281, R302-309.

Uni Z., Gal-Garber O., Geyra A., Sklan D. and Yahav S**.** (2001). Changes in growth and function of chick small intestine epithelium due to early thermal conditioning**.** Poult. Sci. 80, 438-445.

Yahav, S. and McMurtry, J. (2001). Thermotolerance acquisition in broiler chickens by temperature conditioning early in life – the effect of timing and ambient temperature. Poult. Sci. 80, 1662-1666.

Appendix

Table of content

Unpublished data

The effect of thermal conditioning on thermoregulation, metabolic fuel and hormones regulation growth.

Protein concentration in the liver homogenate

In measuring liver 5'mono-deiodinase activity, a very interesting corollary observation was made in that liver protein concentration was greater in thermal conditioned chicks compared to control chicks. At 4 days of age, the liver protein concentration was similar in the TC (thermal conditioned) and control chicks. However, 21 days of age and thereafter, it was higher in the TC chickens and was even significantly higher after the thermal challenge and after 24 h of recovery ($P \leq 0.01$); (Fig. 1). It was well documented that TC chickens undergo compensatory growth after thermal conditioning phase. It coincided with significantly higher feed intake and resulted in higher to significantly higher body weight at marketing age (Yahav, 2000). It can be speculated, therefore, that greater liver development maybe associated with an overall metabolic increase in liver activity coincident with higher protein concentration.

Thyroid hormones

Plasma triiodothyronine (T_3) was significantly greater in thermal conditioned chicks compared to controls during the period of compensatory growth (Day 21-42), and at 18 hours after the end of the challenge phase (Fig. 2). This was in agreement with other reports (Kuhn et al., 1985; McMurtry et al., 1988) in which it had been shown that circulating thyroid hormones were positively correlated to growth or compensatory growth. Blood T_3 was similar in both groups during the challenge phase. The overall pattern of this hormone during the period studied was in agreement with previous results (Yahav and Plavnik, 1999).

Plasma T_4 concentration (Fig. 3) was lower in the TC chickens than in the control one, suggesting a lower activity of the gland in these animals.

Hepatic 5' deiodinase (5'D) activity (Fig. 4) was higher in the TC chickens from 5 days of age till the end of the experiment, except at the end of the challenge (6 hours) when 5'D activity was higher in the control chickens. However, when deiodinase activity was calculated on the basis of activity per gram of tissue, 5'deiodinase activity was significantly higher in TC chickens during the thermal challenge phase (Fig. 4A). The association between T_3 concentrations and 5'D activity is shown in this study in that during the experimental period, both T_3 (Fig. 2) and 5'D activity (Fig. 4A) decreased as the chickens aged, except during heat stress when 5'D activity increased while plasma T_3 decreased. This may be explained by the production of 3,3'5-triiodothyronine (rT_3) during heat exposure. rT_3 is a hypometabolic hormone and antagonizes the hypermetabolic effect of T_3.

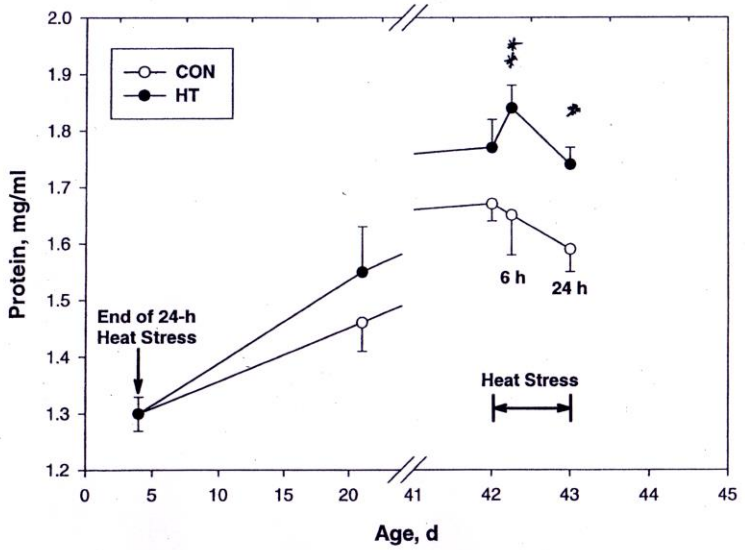


Fig 1: The effect of thermal conditioning, compensatory growth and thermal challenge on liver protein concentration. Values designated by different letters, differ significantly ($P \leq 0.05$). CON = Control group; HT = thermal conditioned group.

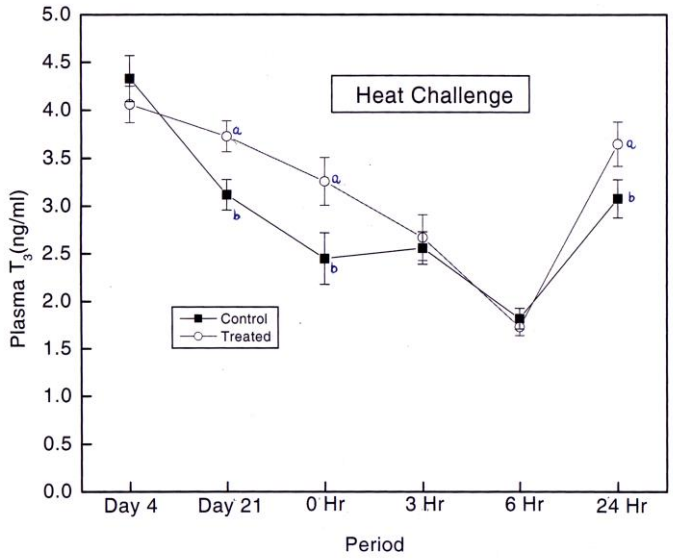


Fig 2: The effect of thermal conditioning, compensatory growth and thermal challenge on plasma triiodothyronine (T_3) concentration. Values designated by different letters, differ

significantly ($P \leq 0.05$). Control = control group; Treated = thermal conditioned group.

Heat stress is accompanied by an increase in rT_3 (Rudas and Pethes, 1984). Both hormones are produced in the liver, T_3 from T_4 by outer-ring deiodination (ORD), whereas inner-ring deiodination (IRD) deiodinate T_4 into rT_3 and T_3 into T_2 (Darras et al., 1992). It can be suggested that during heat stress although the activity of ODR continued to be higher, as we demonstrated, an increase in the IDR reduced the concentration of T_3 by converting it into T_2, or by producing rT_3 from T_4. It can be further hypothesized that the final control of T_3 production is in the liver. This was supported by our results in which T_4 concentrations were suppressed after TC and at other sampling periods, despite changes in T_3 concentrations. It is not evident why a decline in T_3 cannot be explained by the regulation of ORD only, unless T_2 also plays a role. Further studies on the IRD are required.

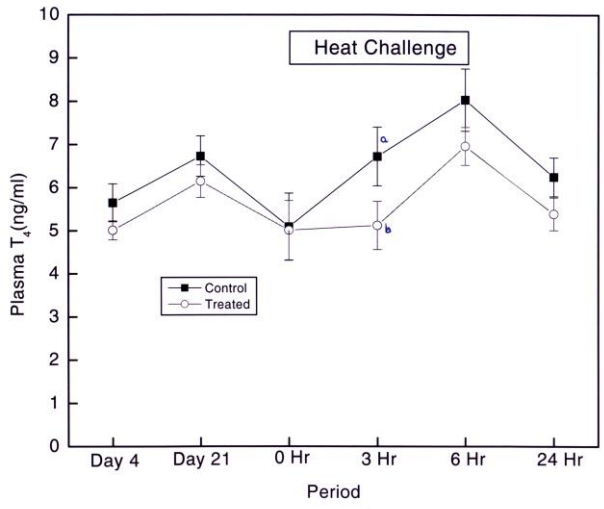
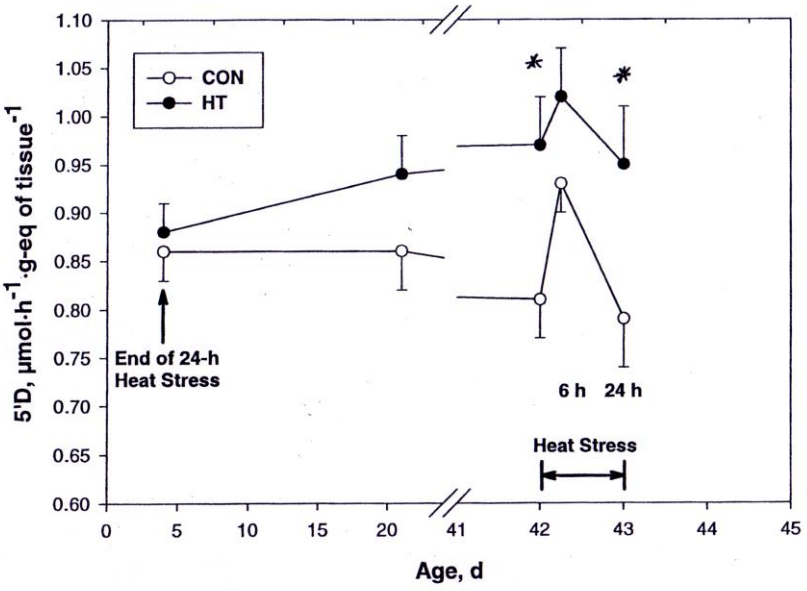


Fig. 3: The effect of thermal conditioning, compensatory growth and thermal challenge on plasma thyroxine (T_4) concentration. Values designated by different letters, differ significantly ($P \leq 0.05$). Control = control group; Treated = thermal conditioned group.

Metabolic rate

Metabolic rate was directly analyzed by measuring oxygen consumption (Fig. 5). During thermal conditioning oxygen consumption was significantly higher in TC chickens, most probably as a result of increased heat loss (mainly by panting).



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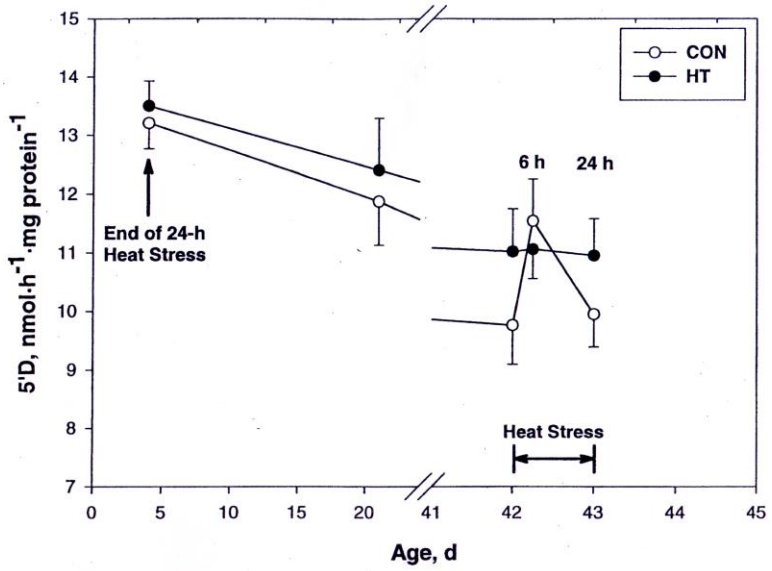


Fig 4: The effect of thermal conditioning, compensatory growth and thermal challenge on liver 5'-Deiodinase activity (per g-eq tissue (upper graph - A), and per mg protein - B, respectively). Values designated by stars, differ significantly ($P \leq 0.05$). CON = Control group; HT = Thermal conditioned group.

During the compensatory growth period metabolic rate was lower in TC chickens than in the controls. This pattern was changed during thermal challenge, where O_2 consumption was slightly higher in TC chickens. It can be speculated that during thermal conditioning at 3 days of age, the threshold response to heat desipation was changed, however, this has to be clarified in further research.

Sensible heat loss

Sensible heat loss was calculated according to surface temperature that was measured accurately by Infrared Thermal Imaging Radiometer. During thermal conditioning, heat loss by radiation and convection was significantly lower in the TC chickens in comparison to

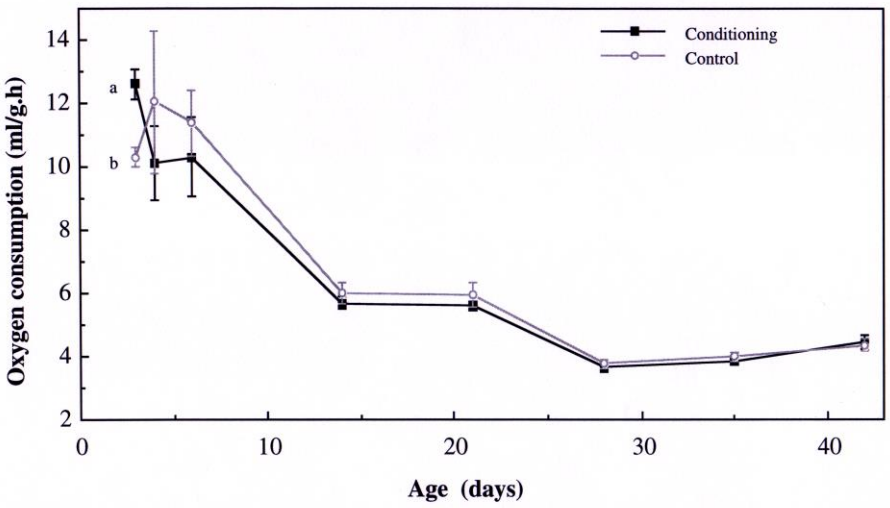


Fig 6: Oxygen consumption of thermal conditioned and control broiler chickens during life span. The values at the age of 42 days are during thermal challenge. Values with different letters differ significantly (P≤0.05). n=6.

controls (P≤0.001) (Table 1). During thermal challenge**,** heat loss by convection was higher in TC chickens whereas that of radiation was significantly higher (P≤0.03).

During thermal conditioning, a significantly lower sensible heat loss in the TC chickens resulted from the small surface to ambient temperature differences.

If TC directly influences vasodilation, and in turn, sensible heat loss in TC chickens during thermal challenge, is not clear. However, the results clearly exhibit an increase in sensible heat loss in the treated chickens during the thermal challenge.

Table 1: The effect of thermal conditioning and challenge on heat loss by radiation and convection of broiler chickens.

Age (days)	Radiation (w)	Convection (w)	Total loss (w)
4 (control)	0.329 ± 0.021^{a}	0.460 ± 0.028^{a}	0.789 ± 0.048^{a}
4 (TC)	0.100 ± 0.023^{b}	0.167 ± 0.030^{b}	0.267 ± 0.052^{b}
42 (control)	1.723 ± 0.181^{b}	3.109 ± 0.279	4.833 ± 0.435

42 (TC)	2.382±0.195[a]	3.532±0.302	5.913±0.470

N=8. Values designated by different letters differ significantly ($P \leq 0.001$; and 0.03 for chickens at the age of 4 and 42 days, respectively).

Evaporative heat loss

Evaporative heat loss was monitored indirectly by measuring the decline in body weight during the measurement of O_2 consumption, taking into account that during the 1-1.5 hours of measurement, most of the reduction in body weight (BW) was from water loss. The decline in BW was 32.15±3.14 and 28.58±5.32 g in control and TC chickens, respectively. These results suggest that the ability of the TC chickens to increase sensible heat loss during thermal challenge, enables them to reduce evaporative heat loss, and thereby, the level of hyperthermia as suggested previously (Yahav and Hurwitz, 1996).

Corticosterone, Glucagon, IGF-I, IGF-II, Insulin, lactate and glucose concentration

Elevated ambient temperature had a clear effect on circulating corticosterone in that concentrations were greater in TC chicks (37.5C) than in control chicks (32C) during thermal conditioning (day 4). Corticosterone levels were significantly elevated in both groups at 6 hrs into the heat challenge phase (37.5C). However, the magnitude of the corticosterone response was blunted in the TC chickens compared to control chicks. ($P \leq 0.053$; Fig. 6).

Plasma glucagon concentrations were the same in both groups until encountering heat stress at 42 days of age (Fig. 7). Within 3 hrs after the initiation of heat challenge, plasma glucagon was significantly ($P<0.05$) elevated, and continued to increase as the challenge phase continued, peaking at 6 hrs. Similar to the corticosterone response, the extent of the glucagon increase was less ($P<0.01$) in the TC chicks compared to control birds. Following the end of heat stress, glucagon concentrations returned to pre-challenge levels.

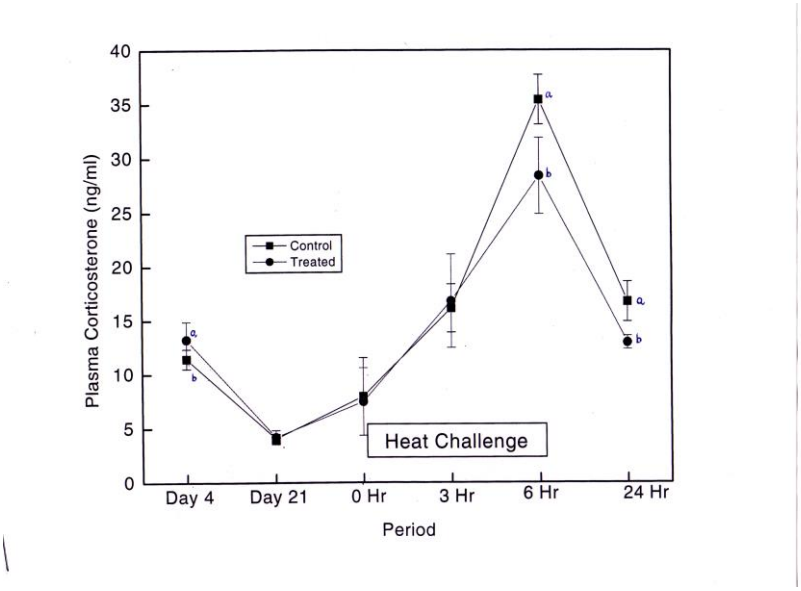


Fig 6: The effect of thermal conditioning (day 4) and thermal challenge (day 42) on plasma corticosterone concentration of control and thermal conditioned chickens. (Values designated by different letters, differ significantly $P<0.05$). Control = control group; Treated = thermal conditioned group.

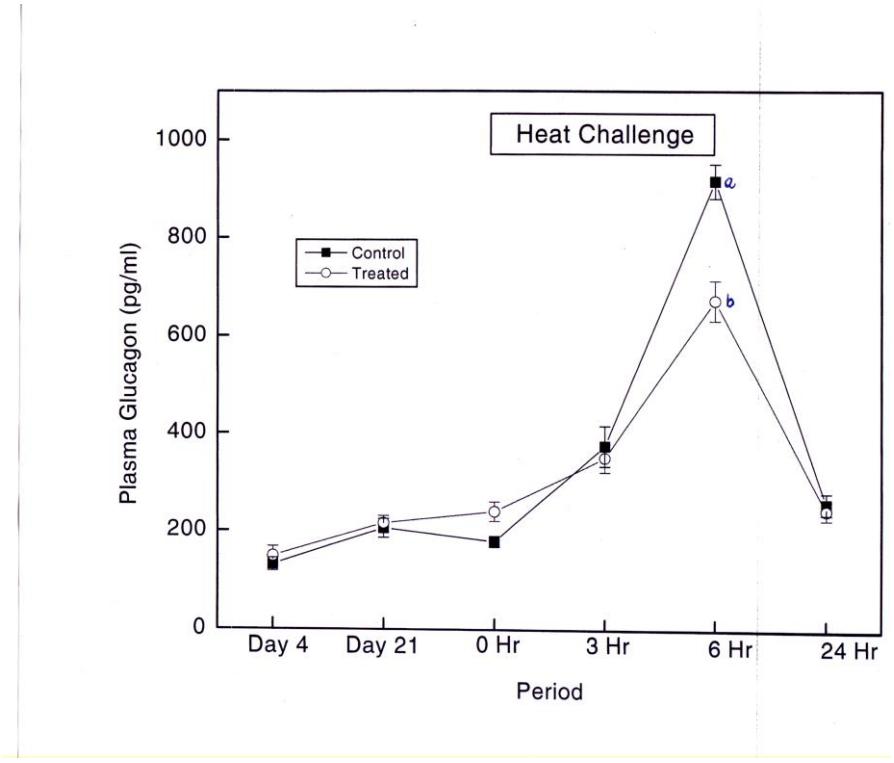


Fig 7: The effect of thermal conditioning, compensatory growth and thermal challenge on plasma glucagon concentration. Values designated by different letters, differ significantly ($P\leq0.05$). Control = control group; Treated = thermal conditioned group.

Circulating insulin levels were similar between groups at all sampling periods. Heat challenge at 42 days of age depressed ($P<0.05$) insulin levels 6 hrs after the onset, and returned to pre-challenge concentrations 18 hrs post-challenge (Fig. 8).

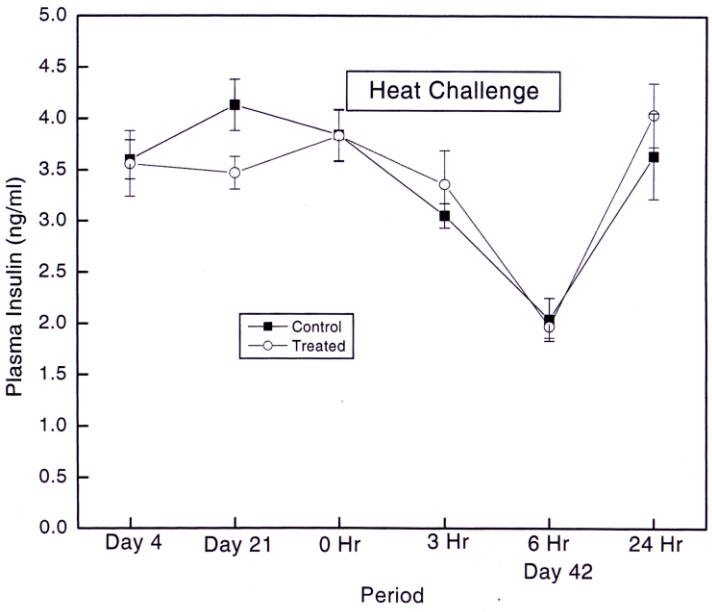


Fig 8: The effect of thermal conditioning, compensatory growth and thermal challenge on plasma insulin concentration. Values designated by different letters, differ significantly ($P\leq0.05$). Control = control group; Treated = thermal conditioned group.

Plasma IGF-I levels were similar between groups at all sampling periods (Fig. 9). Growth factor concentrations increased with advancing age, similar to what was previously reported for growing broiler chickens (McMurtry et al., 1994).

IGF-II concentrations remained unchanged until the heat challenge phase when levels were significantly greater ($P<0.05$) 6 hrs into heat stress (Fig. 10). The extent of the IGF-II response was greater ($P<0.01$) in the control birds compared to their thermal conditioned counterparts.

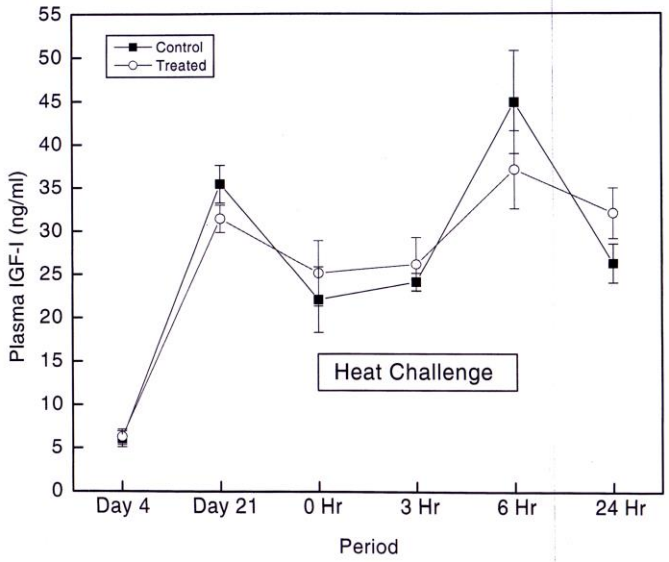


Fig 9: The effect of thermal conditioning, compensatory growth and thermal challenge on plasma IGF-I concentration. Values designated by different letters, differ significantly ($P \leq 0.05$). Control = control group; Treated = thermal conditioned group.

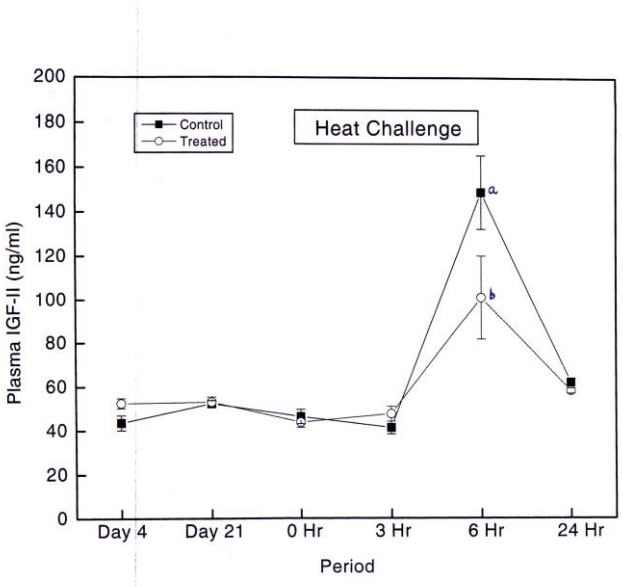


Fig. 10: The effect of thermal conditioning, compensatory growth and thermal challenge on plasma IGF-II concentration. Values designated by different letters, differ significantly ($P \leq 0.05$). Control = control group; Treated = thermal conditioned group.

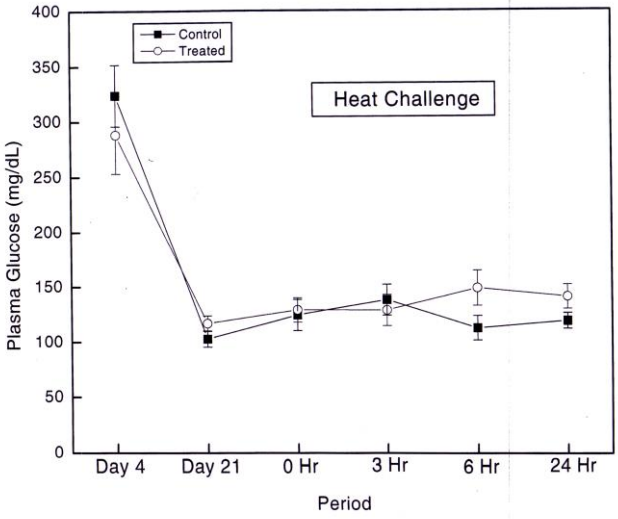


Fig. 11: The effect of thermal conditioning, compensatory growth and thermal challenge on plasma glucose concentration. Values designated by different letters, differ significantly ($P \leq 0.05$). Control = control group; Treated = thermal conditioned group.

Circulating glucose and lactate declined in both groups of chickens with increased age, and was unrelated to any compensatory growth or heat challenge physiological responses (Figs. 11 and 12).

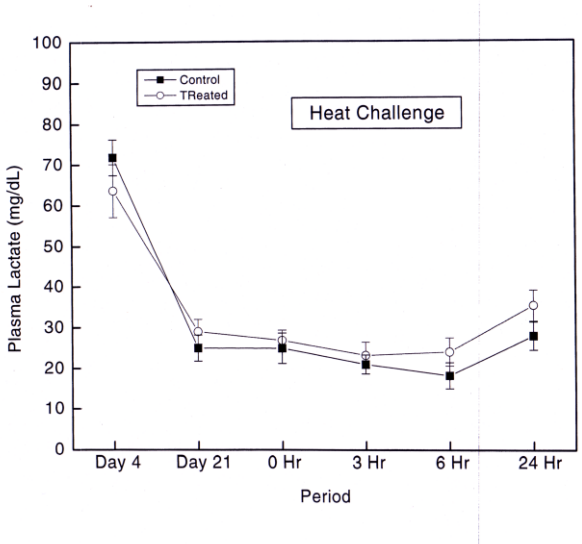


Fig. 12: The effect of thermal conditioning, compensatory growth and thermal challenge on

plasma lactate concentration. Values designated by different letters, differ significantly ($P \leq 0.05$). Control = control group; Treated = thermal conditioned group.

These results in these studies demonstrated a significantly higher stress status in the control chickens during the heat challenge phase compared to thermal conditioned birds. This is based on the corticosterone (Puvadolpirod and Thaxton, 2000), glucagon (Freeman, 1976, 1980, 1982) and IGF II (McMurtry et al., 1998) response to stress. It can be concluded therefore, that the control chickens were under a higher stress condition than the treated chickens, as was also demonstrated by their rate of mortality that amounted during the challenge to 50% compared with 24% of the TC chickens. The significant decline in insulin concentration in both treatments during thermal challenge can be related to its thermogenic effect (Piolino et al., 1990). The ability of the TC chickens to maintain lower body temperature (Yahav and Hurwitz, 1996), to reduce evaporative heat loss, to increase sensible heat loss, suggested a more efficient ability to cope with heat stress as a result of the early in life thermal conditioning.

Gene expression in the hypothalamus and liver of thermal conditioned chickens

(Israel). The objective of the research summarized in this chapter was to identify messenger RNAs (mRNAs) that are induced as a result of thermal conditioning in the hypothalamus. The center for regulation of body temperature control is neuroanatomically located in the preoptic/anterior hypothalamus (PO/AH). This nucleus plays dual functions, both monitoring local temperature changes and integrating temperature information from the periphery. Intrinsic hypothalam**ic** temperature is monitored by temperature sensitive neurons. These neurons change their firing rate in correlation with hypothalamic temperature (Boulant and Dean 1986). They comprise 40% of the PO/AH neurons and are divided into two groups: 1) about 75% of these cells are warm sensitive and their firing rate is increased when temperature is elevated, and decreased if the hypothalamus is cooled**;** 2) about 25% of the temperature sensitive neurons are cold sensitive they exert an opposite profile**,** i.e.**,** their firing rate is increased in the cold and decreased when the hypothalamic temperature is elevated. (Kelso et al. 1982**;** Dean and Boulant 1989a). The adaptation of rats to changed temperature

brought about a change in the ratio between sensitive and insensitive hypothalamic neurons. In control rats approximately 40% of the neurons are warm sensitive. This population was increased to 52% in cold-adapted animals**,** but decreased to 29% in warm adapted rats (Pierau et al. 1998). This change in firing rate, i.e.**,** increase in the rise of depolarizing pre-potentials and a decrease in the interspike interval, is probably due to a change in the opening or closing

rate of potassium channels that participate in the post-hyperpolarization phase of the action potential**,** which is primarily determined by a decrease in outward potassium flow (Dean & Boulant 1989b, Hori et al. 1980 Griffin and Boulant 1995, Griffin et al. 1996). Changes in the ratio between warm, cold and non-sensitive neurons in the PO/AH of Muscovy ducklings as a result of thermal treatment during the prenatal period was also described by Tzschentke and Basta (2002). The main hypothesis being investigated is that thermal protection observed in chickens as a function of thermal conditioning in early life may be based on changes in gene expression patterns.

In order to evaluate the changes in mRNA as a result of the physiological treatment a method of RNA fingerprinting and differential display was established. Using this method, random primers are used to amplify mRNA fragments that can be compared on a sequencing gel. A sample of a gel obtained with an induced gene is depicted in figure 1. The bands that appear to be differentially expressed on the sequencing gel are extracted from the gel and are sequenced either using direct sequencing or by ligation into a plasmid and then sequencing.

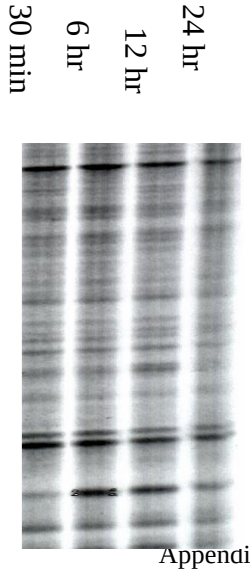


Fig 1. A differential display example of PCR products from hypothalamic mRNA from chicks that where subjected to heat conditioning for 0.3, 6, 12, and 24 hours. The gene indicated with an arrow was induced after 6 hr. This gene was further studied using Northern blot analysis and in situ hybridization.

Cloned and sequenced →

Since differential display is based on an amplification method there might be false positives**,** i.e.**,** genes that seem to be amplified on the sequencing gel**,** but are not amplified. In order to prevent such mistakes each gene was further studied using: 1) a new physiological experiment, 2) PCR using specific primers (primers that are planed according to the sequence obtained from the gel band), 3) northern blot analysis, and 4) in situ hybridization to further study the spatial and temporal pattern of induction.

After screening 15% of the optional mRNAs and verification of the amplification**,** three groups of genes were induced and identified as a result of thermal conditioning acclimation or cold stress: 1) known chick genes, i.e., genes whose sequence has been published in the gene bank, 2) genes with high homology to genes from other species that were published in the gene bank, and 3) new genes. The results of these experiments are summarized in Table 1. Since the working hypothesis was that the genes that are critical for hypothalamic plasticity are genes that are potentially involved with neuronal growth this study focused on gene 14-3-3ε, which has been strongly implicated with brain growth and development. This gene (14-3-3ε) was induced after 24 hours of heat stress (**s**ee figure 2.). The full sequence of the gene 14-3-3**ε** for the chick is currently been analyzed.

Table 1. mRNAs that were induced in the hypothalamus of thermally conditioned chicks

mRNA with known chick sequence	mRNA with high homology to known genes	New genes
Lactate dehydrogenase A	CALM1	Shlo13
Haplotype B cytochrome B	14-3-3ε	Shlo 12
claustrin		2C
Protocadherin 2		3D
Enolase α		2A
		2D

24 h.
12 h.
6 h.
1 h.
Control

Appendix G6a

Fig. 2. PCR products of 14-3-3**ε** separated on a 2% agarose gel and visualized using ethidium bromide. Each band is a result of RNA amplification from the hypothalamus of 5 chicks exposed to thermal conditioning.

(USA). The focus of this research was to identify those genes that impact thermotolerance late in life as a result of early life thermal conditioning. Broiler chicks were, at 3 days of age, either exposed to 37.5˚C for 24 hours or maintained at 32˚C. At 42 days, both groups were

heat challenged at 36.5˚C for 6 hours. Initial experiments centered on tissues collected at the end of the thermal challenge phase for analysis by differential display. RAP-PCR was used to identify genes differentially expressed in the livers of the thermal conditioned and control birds. The same primer is then used in a second strand synthesis/PCR amplification to amplify only those first strand products in a similar fashion. Competition in the amplification step dictates the predominant PCR products present and is template-dependent. Upon separation of the products by electrophoresis differences in the banding pattern can be observed and the individual bands isolated, re-amplified, cloned, sequenced, and identified. Northern analysis can then be used to confirm the pattern of expression.

Differential display-RAP-PCR was performed on total RNA isolated from liver of 6 individuals from both thermal conditioned (H) and control birds-C based on the method of Welsh et al., (1992), using a total of I different arbitrary primers (Fig. 3). The products were separated by denaturing polyacrylamide gel electrophoresis and detected by autoradiography. Examples of amplification products produced by a single primer, indicating differential expression, are shown by the circles (Fig. 3). Selected bands were excised, eluted, and subjected to an additional round of PCR amplification, cloned, and sequenced.

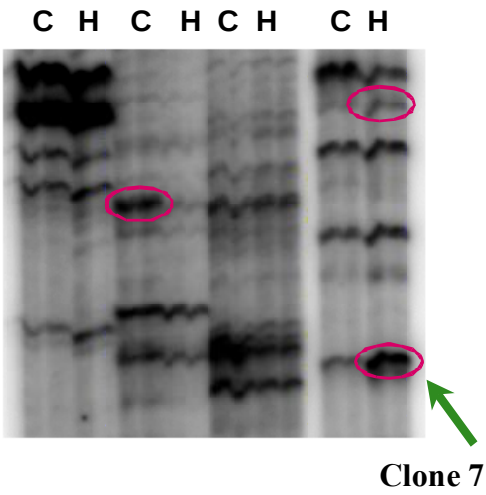
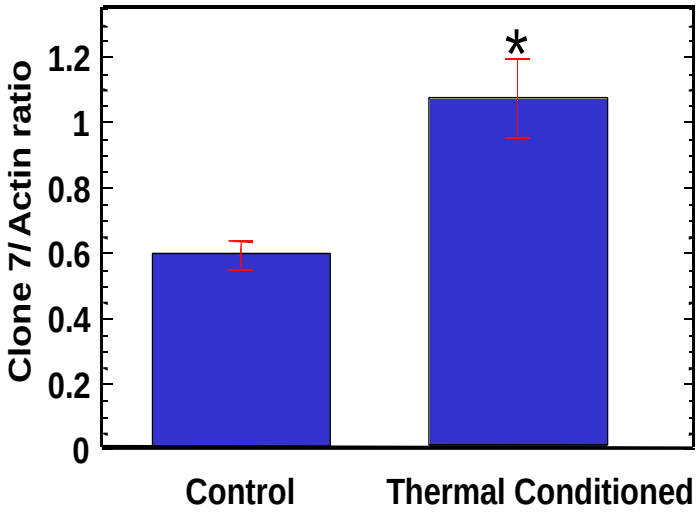


Figure 3 Northern analysis-A Northern blot was prepared by electrophoresis and blotting of 10 μg of total RNA from each of the individuals tested by RAP-PCR and hybridization with random prime labeled prime labeled β-DNA. Random actin cDNA was used as an internal control for normalization.



Total radiolabel hybridized for each transcript was quantified by phosphoimaging and densitometry.

Quantification of clone 7 expression levels between thermal conditioned and control birds is shown in Figure 4 (see below). The average signal present for each mRNA is shown (±stdev). A significant difference is present between the thermal conditioned and control birds (t-test, $P<0.0002$).

Clone 7 classification by sequence comparison – The complete clone 7 sequence was submitted to BLAST search that detected strong homology to several different chicken class II cytokine receptor genes (IL10R2, interleukin 10 receptor 2; INFAR1/INFAR2, interferon alpha/beta receptors 1 and 2, as well as zinc finger gene, ZF 5. The pattern of homology suggests that Clone 7 is a novel class II receptor that consists of domain(s) that are similar (~80% identity) to the IL-10 receptor. These preliminary studies indicate that early life thermal conditioning imparts late life thermo-protection and that there are genes that are potentially affected late in life by early life thermoconditioning. The observation that clone7, a novel class II cytokine receptor is differentially expressed between the control and thermal conditioned birds during thermal stress may indicate some protective role.

This novel class II cytokine receptor, like IL10R, may function as an anti-inflammatory mediator and this imparts resistance to thermal stress. It is presently unknown how this gene is expressed during thermal conditioning or otherwise throughout normal development. These indicative data require further investigation.

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